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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 March 2007 (29.03.2007)

PCT

(10) International Publication Number
WO 2007/035961 A2

(51) International Patent Classification:
A61K 31/7072 (2006.01)

(21) International Application Number:
PCT/US2006/037431

(22) International Filing Date:
25 September 2006 (25.09.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/719,787	23 September 2005 (23.09.2005)	US
60/760,599	20 January 2006 (20.01.2006)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

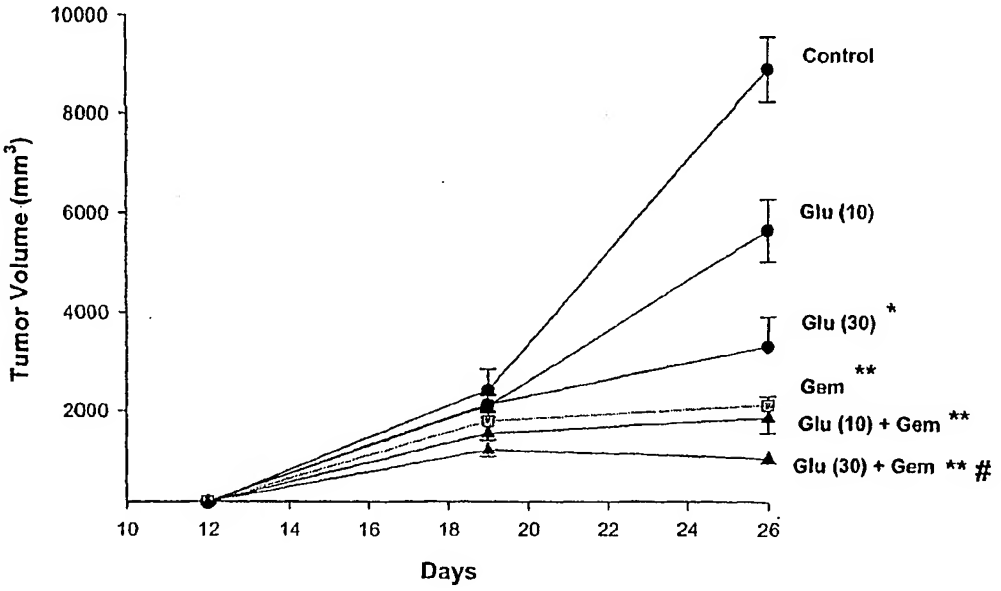
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTI-CANCER THERAPIES



(57) Abstract: Cancer can be treated by administration of glufosfamide alone or in combination with another anticancer agent.

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ANTI-CANCER THERAPIES

RELATED APPLICATIONS

This application claims the benefit of U.S. Patent Application Nos. 60/719787, filed 23 September 2005, and 60/760599, filed 20 January 2006, the contents of which are incorporated herein by reference.

TECHNICAL FIELD

The present invention provides compositions and methods for the treatment of cancer and so relates to the fields of medicine and pharmacology.

BACKGROUND OF THE INVENTION

"Cancer" refers generally to one of a group of more than 100 diseases caused by the uncontrolled, abnormal growth of cells that can spread to adjoining tissues or other parts of the body. Cancer cells can form a solid tumor, in which the cancer cells are massed together, or exist as dispersed cells, as in leukemia. Normal cells divide (reproduce) until maturation is attained and then only as necessary for replacement of damaged or dead cells. Cancer cells are often referred to as "malignant", because they divide endlessly, eventually crowding out nearby cells and spreading to other parts of the body. The tendency of cancer cells to spread from one organ to another or from one part of the body to another distinguishes them from benign tumor cells, which overgrow but do not spread to other organs or parts of the body. Malignant cancer cells eventually metastasize and spread to other parts of the body via the bloodstream or lymphatic system, where they can multiply and form new tumors. This sort of tumor progression makes cancer a deadly disease. Although there have been great improvements in the diagnosis and treatment of cancer, many people die from cancer each year, and their deaths are typically due to metastases and cancers that are resistant to conventional therapies. Current methods for treatment of advanced and/or metastatic malignancies previously treated with chemotherapy (*i.e.* chemotherapy-refractory cancers) are inadequate. Curative therapy is rarely possible in patients with advanced malignancies that have relapsed after chemotherapy.

There is a need in the art for improved methods for the treatment of advanced and/or metastatic malignancies previously treated with chemotherapy. The present invention addresses these needs and provides compositions and methods for treating cancer.

SUMMARY OF THE INVENTION

The present invention provides methods and compositions for the treatment of cancer. In particular, the invention relates to administration of glufosfamide alone or in combination with another anticancer agent for the treatment of cancer. Further, the present invention provides methods for the treatment of advanced and/or metastatic malignancies, including those previously treated with chemotherapy.

In a first aspect, the present invention provides a method of treating cancer, which method comprises administering a therapeutically effective amount of glufosfamide alone or in combination with gemcitabine or another anti-cancer agent to a subject in need of such treatment. A variety of cancers can be treated by this method, for example breast cancer, colorectal cancer, ovarian cancer, lung cancer, and pancreatic cancer.

In accordance with the methods of the invention, and regardless of whether glufosfamide is administered alone or in combination with another anti-cancer agent such as gemcitabine, glufosfamide is administered at least once per treatment cycle for at least 1, 2, 3, 4, 5, 6, 7, 8, or more than 8 treatment cycles, wherein each cycle is at least one week and can be up to three or four weeks or as long as seven weeks, and in each administration of glufosfamide, glufosfamide is administered to the cancer patient by intravenous infusion in an amount ranging from 1.5 to about 6.0 g/m^2 (g is grams of glufosfamide and m^2 is the surface area of the patient). In one embodiment, the amount of glufosfamide administered ranges from about 1.5 to about 4.5 g/m^2. In one embodiment, the amount of glufosfamide administered is 4.5 g/m^2. In one embodiment, the amount of glufosfamide administered is 5.0 g/m^2. The glufosfamide infusion can be administered over a period 1, 2, 3, 4, 5, or 6 hours. In one embodiment, the glufosfamide is administered once every three or four weeks (the treatment cycle time) at a dose of 4.5 g/m^2 over an infusion time of 1, 4, or 6 hours. In other embodiments, glufosfamide is administered (a) in an amount ranging from 1.5 to about 3.0 g/m^2 (sometimes ranging from 2.0 to about 3.0 g/m^2) over an infusion period of 1-6 hours for three consecutive days (days 1, 2, and 3) every three weeks; (b) 1.5 to about 3.0 g/m^2 (sometimes 2.0 to about 3.0 g/m^2) over an infusion period of 1-6 hours once per week; or (c) 1.5 to about 6.0 g/m^2 (sometimes 4.5 to about 6.0 g/m^2, sometimes about 5.0 g/m^2) over an infusion period of 1-6 hours once every four weeks.

In one embodiment, glufosfamide is administered so that the glufosfamide concentration in the patient's blood plasma or C_{max} reaches at least 50 micrograms/mL, or at least 100 micrograms/mL, or at least 150 micrograms/mL, or at least 250 micrograms/mL. In one embodiment, these blood plasma levels of glufosfamide are reached by administering glufosfamide in amounts ranging from about 1.5 to about 4.5 g/m^2 (sometimes 2.5 to about 4.5 g/m^2) over an infusion period of 1 to 4 hours. In one embodiment, the amount of glufosfamide

administered is 5.0 g/m^2. In one embodiment, glufosfamide is administered so that the glufosfamide area under the concentration-time curve (AUC) is at least 200 micrograms-h/mL, or at least 500 micrograms-h/mL, or at least 1000 micrograms-h/mL. In one embodiment, these blood plasma levels of glufosfamide are reached by administering glufosfamide in amounts ranging from about 1.5 to about 4.5 g/m^2 (sometimes 2.5 to about 4.5 g/m^2), respectively, over an infusion period of 1 to 4 hours.

Glufosfamide is a pro-drug that is converted into the active metabolite isophosphoramide mustard (IPM) *in vivo*, and in one embodiment, glufosfamide or another IPM pro-drug or IPM itself (which is typically co-administered with MESNA to avoid harmful side effects, as is known in the art) is administered so that the IPM concentration in the patient's blood plasma reaches at least 1 microgram/mL, or at least 3 micrograms/mL, or at least 4 micrograms/mL. In one embodiment, these blood plasma levels of IPM are reached by administering glufosfamide in amounts ranging from about 1.5 to about 4.5 g/m^2 (sometimes 3.5 to about 4.5 g/m^2), respectively, over an infusion period of 4 hours. In one embodiment, glufosfamide or another IPM pro-drug or IPM itself is administered so that the IPM AUC is at least 10 micrograms-h/mL, or at least 25 micrograms-h/mL, or at least 35 micrograms-h/mL. In one embodiment, these blood plasma levels of IPM are reached by administering glufosfamide in amounts ranging from about 1.5 to about 4.5 g/m^2 (sometimes 2.5 to about 4.5 g/m^2), respectively, over an infusion period of 1 to 4 hours. In one embodiment, these blood plasma levels of IPM are reached by administering glufosfamide at 5.0 g/m^2.

In one embodiment, glufosfamide is administered as described above and gemcitabine is also administered for 1, 2, 3, 4, 5, 6, 7, 8 or more than 8 of the treatment cycles, and each administration of gemcitabine in each treatment cycle comprises an intravenous infusion of gemcitabine in a dose ranging from about 1000 mg/m^2 to about 2200 mg/m^2 (sometimes from about 1000 mg/m^2 to about 1500 mg/m^2; and sometimes about 1500 mg/m^2 to about 2200 mg/m^2) over an infusion time ranging from about 30 to about 150 min. In one embodiment in which glufosfamide and gemcitabine are co-administered, gemcitabine is administered on weeks 1, 2, 3, 5, 6, and 7 of a treatment cycle for 1, 2, 3, 4, or more than 4 treatment cycles, wherein each cycle is a seven-week cycle. In one embodiment, gemcitabine is administered on weeks 1, 2, and 3 of a treatment cycle for 1, 2, 3, 4, 5, 6, or more than 6 treatment cycles, wherein each cycle is a four-week cycle. Gemcitabine can be administered in accordance with the methods of the invention one day before, one day after, or on the same day as, the administration of glufosfamide. In one embodiment, gemcitabine is administered on the same day as the administration of glufosfamide, about 30 minutes to about 4 hours after the glufosfamide is administered.

In one embodiment in which glufosfamide and gemcitabine are co-administered, gemcitabine is administered so that the gemcitabine concentration in the patient's blood plasma reaches at least 20 micrograms/mL. In one embodiment, this blood plasma level of gemcitabine is reached by administering gemcitabine in an amount of about 1 g/m^2, over an infusion period of about one-half hour. Gemcitabine is a pro-drug that is converted into the active metabolite dFdU *in vivo*, and in one embodiment, gemcitabine is administered so that the dFdU concentration in the patient's blood plasma reaches at least 30 micrograms/mL. In one embodiment, this blood plasma level of dFdU is reached by administering gemcitabine in an amount of about 1 g/m^2 over an infusion period of one-half hour.

In a second aspect, the present invention provides a method of treating cancer, which method comprises administering glufosfamide to a subject in need of treatment of advanced and/or metastatic malignancies previously treated with chemotherapy. In one embodiment, the present invention provides methods for the treatment of a gemcitabine-refractory pancreatic cancer.

In a third aspect, the invention provides a method for treating cancer, comprising administering an agent with antitumor activity, such as bevacizumab (Avastin®), carboplatin, cetuximab (Erbitux®), cisplatin, dacarbazine (DTIC), 2-deoxyglucose, doxorubicin (*e.g.*, Doxil® and Caelyx®), EGFR inhibitors (e.g., Iressa), erlotinib (Tarceva®), etoposide, exatecan, imatinib mesylate (Gleevec®), irinotecan, methotrexate, Panvac-VF®, pemetrexed (Alimta®), rituximab, rubitecan (Orathecin®), taxanes (*e.g.*, docetaxel and paclitaxel), topotecan, vincristine, or trastumab (Herceptin®), in combination with glufosfamide or in combination with glufosfamide and gemcitabine or in combination with glufosfamide, gemcitabine, and erlotinib, to a patient in need of such treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effects of glufosfamide and gemcitabine in proliferation assays. MiaPaCa-2 (A), Hst-766 (B), and AsPC-1(C) pancreatic cells were grown in culture media for three days as described in the Examples. Glufosfamide and gemcitabine were added to the media at the indicated concentrations alone or together and cell numbers determined daily with a hemocytometer.

Figure 2 shows an analysis of DNA fragmentation by FACS. 1 X 10^6 cells were incubated with vehicle (A), gemcitabine (B), glufosfamide (C), or the combination of both agents (D) at 10 μg/mL for 24 hours with addition of propidium iodide.

Figure 3 shows final tumor volumes (A) and 50% survival (B) recorded in the MiaPaCa-2-RFP pancreatic cancer model. Glufosfamide was administered IV daily for 14 days. * means $P < 0.05$ vs control.

Figure 4 shows primary tumor volumes recorded in the MiaPaCa-2-RFP pancreatic cancer model. Gemcitabine (IP, once a week for three weeks) and glufosfamide (IV daily for 14 days) each significantly reduced tumor volume measured on day 26 but the combination of 30 mg/kg and gemcitabine resulted in significantly greater reduction. * means $P < 0.05$ vs control; ** means $P < 0.01$ vs control; # means $P < 0.05$ vs gemcitabine alone.

Figure 5 shows the apoptotic index and proliferative index measured by TUNEL and PCNA stains. Tumors were removed at the end of treatment and processed for IHC. * means $P < 0.05$ vs control; ** means $P < 0.01$ vs control; *** means $P < 0.001$ vs control; # means $P < 0.05$ vs gemcitabine.

Figure 6 shows the plasma concentration of glufosfamide (μg/mL) at 0, 4, 8, 12, 16, 20, and 24 hours after the start of glufosfamide infusion on day 1 of a treatment cycle at various doses (1500, 2500, 3500 and 4500 mg/m^2) of glufosfamide.

Figure 7 shows the plasma concentration of glufosfamide metabolite isophosphoramide mustard (IPM) (μg/mL) at 0, 4, 8, 12, 16, 20, and 24 hours after the start of glufosfamide infusion on day 1 of a treatment cycle at various doses (1500, 2500, 3500 and 4500 mg/m^2) of glufosfamide.

Figure 8 shows the plasma concentration of gemcitabine (μg/mL) at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 hours after the start of gemcitabine infusion on days 1 and 8 of a treatment cycle at 1000 mg/m^2 of gemcitabine.

Figure 9 shows the plasma concentration of gemcitabine metabolite dFdU (μg/mL) at 0, 4, 8, 12, 16, 20 and 24 hours after the start of gemcitabine infusion on days 1 and 8 of a cycle at 1000 mg/m^2 of gemcitabine.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect of the invention, glufosfamide and optionally gemcitabine and optionally one or more additional anti-cancer agents are administered in therapeutically effective amounts to a subject in need of treatment for cancer.

The antitumor drug glufosfamide (ß-D-glucosyl-isophosphoramide mustard; glc-IPM) is an alkylating agent that is being investigated for use in the treatment of cancer (see U.S. Patent No. 5,622,936, incorporated herein by reference, and Niculescu-Duvaz, 2002, *Curr Opin Investig Drugs* 3:1527-32). The alkylating moiety (isophosphoramide mustard, IPM) is glycosidically linked to ß-D-glucose, and cellular uptake of glufosfamide is believed to be mediated by a sodium-dependent trans-membrane glucose transporter protein (Briasoulis *et al.*, 2000, *J Clin Oncol* 18:3535-44) and other glucose transporters, as described herein. In Phase II clinical studies, glufosfamide has been administered to patients with pancreatic cancer receiving first line treatment and in patients with non-small cell lung cancer receiving second line chemotherapy, as well as glioblastoma, breast cancer, and colon cancer patients (see Niculescu-Duvaz, 2002, *supra*). Glufosfamide is administered intravenously; it is contemplated that in the practice of the present invention other administration routes may also be used, such as intrathecal administration, intratumoral injection, oral administration, and other routes.

In one embodiment of the invention, gemcitabine is co-administered with glufosfamide. Gemcitabine (2'-deoxy-2',2'-difluoro-cytidine, also known as 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose) is a nucleoside analogue that disrupts the process of cell replication. See U.S. Pat. Nos. 4,808,614 and 5,464,826, each of which is incorporated herein by reference. Gemcitabine HCl (Gemzar™; Eli Lilly) has been used for treatment of patients with non-small cell lung cancer and pancreatic cancer. Gemcitabine HCl is routinely formulated as a sterile solution and is administered by intravenous infusion. Other salt forms, *e.g.*, the monophosphate, sulfate, malonate, citrate, and succinate are readily prepared, and can be utilized if desired. It is contemplated that other administration routes can be used, including intratumor injection, intrathecal administration, and others. As noted above, in certain embodiments of the present invention, gemcitabine is co-administered with glufosfamide to treat a cancer patient.

As used herein, "a subject" or "patient" is a mammal in need of treatment for cancer. Generally, the subject is a human cancer patient. In some embodiments of the invention, the subject is a non-human mammal used in a model system (*e.g.*, a non-human primate, mouse or rat used in screening, characterization, and evaluation of medicaments).

As used herein, and as is well-understood in the art, "treatment" is an approach for obtaining beneficial or desired medical results, including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total). "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment or if receiving a different treatment. As provided by the present invention, glufosfamide can be administered alone or in combination with other anti-cancer agents to provide an efficacious treatment of cancer.

As used herein, and as is well-understood in the art, "a therapeutically effective amount" of a drug is an amount of a drug that, when administered to a subject with cancer, will have beneficial or desired medical results, including clinical results, such as described above in the description of treatment. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations.

Two (or three or more) drugs are administered to a subject "in combination" when the drugs are administered as part of the same course of therapy. A course of therapy, in this context, refers to administration of a combination of drugs believed to work together additively, complementarily, synergistically, or otherwise to produce a more favorable outcome than that anticipated for administration of any drug in the combination if administered as a single agent. A course of therapy can be for one or a few days, but more often for cancer drugs, extends for several weeks and for multiple treatment cycles, each of which lasts from a week to a month or more, as described below.

Thus, an example of administration in combination, for illustration and not limitation, is the administration in accordance with the methods of the invention of glufosfamide once every three weeks for at least 2 and up to 8 or more three-week treatment cycles, where the first administration of glufosfamide is defined as beginning on day 1, together with the administration of gemcitabine once each week for weeks 1, 2, 3, 5, 6, and 7 of a seven-week cycle for one or more seven-week cycles, where the administration of gemcitabine begins on day 1, day -1 (one day before day 1), or day 2. Another example of administration in combination is administration of glufosfamide once every four weeks beginning on week 1 (on day 1) of a four-week treatment cycle for 2 to 8 or more four-week cycles, and administration of gemcitabine on weeks 1, 2, and 3 (on days 1, 8, and 15) of the same four-week cycle for 2 to 8 or more four-week cycles. In one

embodiment, gemcitabine is administered on the same day as glufosfamide between about 30 minutes to about 4 hours after the administration of glufosfamide.

When two or more drugs are administered in combination, a variety of schedules can be used. In one case, for example and without limitation, Drug 1 is first administered prior to administration of Drug 2, and treatment with Drug 1 is continued throughout the course of administration of Drug 2; alternatively Drug 1 is first administered after the initiation of Drug 2 therapy; alternatively, Drug 1 is first administered contemporaneously with the initiation of the other (Drug 2) cancer therapy. As used in this context, "contemporaneously" means the two drugs are administered the same day or on consecutive days.

Although in principle certain drugs can be co-formulated and delivered in combination, combination therapies, in general, involve the administration of drugs formulated in separate compositions. Similarly, although certain drugs can be administered simultaneously, more often (especially for drugs administered by intravenous infusion) drugs are administered at different times on the same day, on consecutive days, or according to another schedule. For example, in one embodiment of the present invention, glufosfamide is administered over a 4 hour infusion period once every four weeks beginning on day 1 of a four-week cycle, and gemcitabine is administered over a 30 minute infusion period on days 1, 8 and 15 of the same four-week cycle, and administration of both drugs is continued for 1 to 8 or more four-week cycles. On day 1, gemcitabine is administered 30 minutes after the administration of glufosfamide.

In other embodiments of the invention, glufosfamide is administered as a "single agent;" *i.e.*, not in combination with another antitumor drug. For example, glufosfamide can be administered to treat advanced and/or metastatic malignancies previously treated with chemotherapy, in which method glufosfamide can be administered as a single agent, in combination with one or more of the previously used drugs, or in combination with other drugs with which the patient has not yet been treated. In another embodiment, the invention provides a method of treating locally advanced unresectable or metastatic pancreatic adenocarcinoma previously untreated with chemotherapy, and in this embodiment, glufosfamide may be administered as a single agent or in combination with other anti-cancer drugs. In another embodiment, the invention provides a method treating gemcitabine-refractory metastatic pancreatic adenocarcinoma, and again, in this embodiment, glufosfamide may be administered as a single agent or in combination with gemcitabine, or in combination with another drug used to treat pancreatic cancer.

The methods of the present invention can be used for treatment of any cancer, including but not limited to adenocarcinoma; adenoma; basal cell carcinoma, bone cancer, brain cancer, including primary brain tumors; breast cancer; cancer of the adrenal, bronchi, colon, gallbladder,

head and neck, kidneys larynx, neural tissue, parathyroid, rectum, stomach, and thyroid; cervical dysplasia and *in situ* carcinoma; epidermoid carcinomas; Ewing's sarcoma; giant cell tumor; glioblastoma multiforma; hairy-cell tumor; hyperplasia; hyperplastic corneal nerve tumor; intestinal ganglioneuroma; islet cell carcinoma; leiomyoma; leukemia; liver cancer; lung cancer; lymphocytic and granulocytic tumors, both acute and chronic; lymphomas; malignant carcinoid; malignant hypercalcemia; malignant melanomas; marfanoid habitus tumor; medullary carcinoma; mucosal neuroma; mycosis fungoides; myeloma; neuroblastoma; osteosarcoma; ovarian cancer and tumors; pancreatic cancer; pheochromocytoma; polycythermia vera; prostate cancer; renal cell tumor; retinoblastoma; sarcomas, including Kaposi's sarcoma, osteogenic and other sarcoma, rhabdomyosarcoma, and soft tissue sarcoma; seminoma; skin cancer, including metastatic skin carcinoma and topical skin lesions; small-cell lung cancer; squamous cell carcinoma of both ulcerating and papillary type; veticulum cell sarcoma; and Wilm's tumor.

The methods of the invention are particularly efficacious in the treatment of particular cancers, including breast cancer, colorectal cancer, gall bladder cancer, non-Hodgkin's lymphoma, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, sarcoma, and stomach cancer.

In one embodiment of the invention, the subject to whom glufosfamide treatment is administered has breast cancer. Breast cancer is commonly treated by various combinations of surgery, radiation therapy, chemotherapy, and hormone therapy. In accordance with the methods of the invention, glufosfamide is administered alone, or in combination with gemcitabine, or in combination with another anti-cancer drug for the treatment of breast cancer.

In one embodiment of the invention, glufosfamide is administered to a subject in need of treatment for colorectal cancer. In certain embodiments, the patient to whom treatment is administered has colorectal cancer or metastatic colorectal cancer. Colorectal cancer or metastatic colorectal cancer is currently treated by radiation therapy, surgery, and/or chemotherapy (*e.g.*, administration of 5-fluorouracil). In one embodiment, glufosfamide is administered as a single agent to treat colorectal cancer; in another embodiment, glufosfamide is co-administered with gemcitabine to treat colorectal cancer. In another embodiment, glufosfamide and bevacizumab are administered in combination to a subject in need of treatment for colorectal cancer; optionally, gemcitabine or 5-fluorouracil is also co-administered.

In one embodiment of the invention, glufosfamide is administered as a second-line therapy for non-Hodgkins lymphoma after CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) therapy has failed.

In one embodiment of the invention, glufosfamide is administered to a subject in need of treatment for ovarian cancer either as a single agent or in combination with gemcitabine. In one

embodiment, glufosfamide is administered, either as a single agent or in combination with gemcitabine, as a second line therapy in the treatment of ovarian cancer, optionally in combination with an approved second line therapy after failure on cis/carboplatin and/or a taxane or other first line therapy. In another aspect, glufosfamide is administered as a first line therapy in the treatment of ovarian cancer alone or in combination with gemcitabine, and/or cis/carboplatin and/or a taxane and/or another first line therapy.

In one embodiment of the invention, the subject to whom glufosfamide treatment is administered has pancreatic cancer. Among pancreatic cancers, chemotherapy-refractory pancreatic cancers, such as pancreatic cancers refractory to treatment with gemcitabine (see, *e.g.*, Araneo *et al.*, 2003, *Cancer Invest.* 21:489-96; Kozuch *et al.*, 2001, *The Oncologist* 6:488-95; Noble and Goa, 1997, *Drugs* 54: 447-72N; Stephens *et al.*, 1998, *Oncol. Nurs. Forum* 25:87-93; Burris and Storniolo, 1997, *Eur. J. Cancer* 33: Suppl 1:S18-22; Rothenberg *et* al., 1996, *Ann. Oncol.* 7:347-53), can be treated using the methods disclosed herein, *e.g.*, by administration of glufosfamide alone or in combination with gemcitabine.

In one embodiment of the invention, glufosfamide is administered as a first- or second-line therapy to treat a PET-positive sarcoma. A "PET-positive" sarcoma is a sarcoma detectable by positron emission tomography. In one embodiment, glufosfamide is administered as a second line therapy in the treatment of a sarcoma either alone or in combination with another approved second line therapy after treatment failure on doxorubicin or failure on another first line therapy. In another embodiment, glufosfamide is administered as a first line therapy in the treatment of a sarcoma in combination with doxorubicin or in combination with another approved first line therapy.

In one embodiment of the invention, glufosfamide is administered as a second line therapy in small-cell lung cancer (SCLC) either alone or in combination with gemcitabine and optionally with another approved second line therapy after first line failure on carboplatin or cisplatin, VP16, and/or a taxane. In another aspect, glufosfamide is administered alone or in combination with gemcitabine as a first line therapy in the treatment of SCLC, optionally in combination with carboplatin or cisplatin, VP16, and/or a taxane or other approved first line therapy.

As will be apparent from the foregoing, in certain embodiments of the invention, another anticancer agent is co-administered with glufosfamide to treat cancer. For these embodiments, particularly preferred anti-cancer agents include those selected from the group consisting of bevacizumab (Avastin®), carboplatin, cetuximab (Erbitux®), cisplatin (cis-diaminedichloro-platinum (II), a divalent inorganic water soluble platinum containing complex; see Go and Adjei, 1999, *J Clin Oncol.* 17:409-22), dacarbazine (DTIC), 2-deoxyglucose, doxorubicin (*e.g.*,

Doxil® and Caelyx®), EGFR inhibitors (e.g., Iressa®), erlotinib (Tarceva®), etoposide, exatecan, imatinib mesylate (Gleevec®), irinotecan, methotrexate, Panvac-VF®, pemetrexed (Alimta®), rituximab, rubitecan (Orathecin®), taxanes (*e.g.*, docetaxel and paclitaxel), topotecan, vincristine, or trastumab (Herceptin®). In various embodiments of the invention, one or more of these agents is administered in combination with glufosfamide or in combination with glufosfamide and gemcitabine to a subject in need of treatment for cancer. In one embodiment, the cancer is pancreatic cancer. In one example, for illustration and not limitation, glufosfamide and erlotinib (Tarceva™) and optionally gemcitabine can be co-administered in accordance with the methods of the invention to treat cancer.

Gemcitabine, bevacizumab, irinotecan, exatecan, pemetrexed, cisplatin, and erlotinib, as well as the other anti-cancer agents described herein, can be administered at any dose that is therapeutically effective, such as doses comparable or identical to those routinely utilized clinically. Specific dose regimens for known and approved antineoplastic agents (*e.g.*, the recommended effective dose) are known to physicians and are provided, for example, in the product descriptions found in the PHYSICIANS' DESK REFERENCE, 2003, 57th Ed., Medical Economics Company, Inc., Oradell, N.J; Goodman & Gilman's THE PHARMACOLOGICAL BASIS OF THERAPEUTICS" 2001, 10th Edition, McGraw-Hill, New York; and/or are available from the Federal Drug Administration and/or are discussed in the medical literature. Brief descriptions of certain of these other anti-cancer agents are provided below.

Bevacizumab (Avastin™; Genentech) as used in the present invention can be administered at doses comparable to those routinely utilized clinically for other purposes (see, *e.g.*, Yang, 2003, *N. Eng. J. Med.*, 349:419-21 and Cobleigh *et al.*, 2003, *Semin Oncol.* 30(5 Suppl 16):117-24). Bevacizumab is an anti-vascular endothelial growth factor (VEGF) monoclonal antibody that has been developed as an anti-angiogenesis agent for treatment of cancers such as colorectal cancer, non-small-cell lung cancer, breast cancers, and other solid tumors. *See* Salgaller, 2003, *Curr Opin Mol Ther*, 5:657-67 and PCT applications WO 96/30046, and WO 98/45331. In one embodiment of the present invention, Bevacizumab in combination with glufosfamide is administered to treat such cancers.

Erlotinib as used in the present invention can be administered in a dose ranging from about 25 mg to about 200 mg. In one embodiment, erlotinib is administered in a dose ranging from about 100 mg to about 200 mg. In one embodiment, the dose of erlotinib administered in combination with glufosfamide is about 150 mg. Erlotinib is typically administered at least one hour after the ingestion of food.

Exatecan mesylate (DX-8951f; Daiichi Pharmaceutical Co.) is a water soluble analogue of the plant alkaloid camptothecin that inhibits topoisomerase I. Exatecan mesylate has been

developed as a therapeutic agent for the treatment of non-small cell lung cancer, ovarian, tubal or peritoneal cancer, and breast cancer. Various dosages and administrations of exatecan mesylate for the treatment of cancers have been described. See, *e.g.*, Verschraegen et al, 2004, *Cancer Chemother Pharmacol.* 53:1-7; Esteva *et al.*, 2003, *Cancer* 98:900-7; Braybrooke *et al.*, 2003, *Lung Cancer*, 41:215-9; Royce *et al.*, 2004, *Invest New Drugs.* 22:53-61. In one embodiment of the present invention, Exatecan mesylate in combination with glufosfamide is administered to treat cancers such as non-small cell lung cancer, ovarian, tubal or peritoneal cancer, and breast cancer.

Irinotecan (CPT-11, Camptosar®; Pharmacia & Upjohn) is a semisynthetic derivative of the plant alkaloid camptothecin that inhibits topoisomerase I. It has been developed as an anti-cancer drug for the treatment of colorectal cancer. For purposes of the present invention, Irinotecan can be administered at doses comparable to those routinely utilized clinically. For example, and without limitation, patients can receive Camptosar® in a 90-minute infusion once every 3 weeks. The starting dose for most patients can be 350 mg/m^2, but the dose may decrease to 300 mg/m^2 for patients 70 years of age or older. Camptosar® can also be administered according to a weekly dosing schedule starting at 125 mg/m^2. The dose can be given for about 2 to 4 weeks, with each course of therapy repeated every 7 weeks. See Rothenberg *et al.*, 1996, *J Clin Oncol.*14:1128-35.

Pemetrexed (Alimta™) is an antifolate that inhibits thymidylate synthase, dihydrofolate reductase, glycinamide ribonucleotide formyltransferase, and aminoimidazole carboxamide ribonucleotide formyltransferase. Pemetrexed is active against pancreatic cancer cell lines *in vitro* and has shown activity in patients with advanced pancreatic cancer. See Kindler, 2002, *Semin Oncol.* 29:49-53 and Adjei, 2003, *Expert Rev Anticancer Ther.* 3:145-56. In one embodiment of the present invention, glufosfamide is administered in combination with Pemetrexed and optionally gemcitabine to treat pancreatic cancer.

In another aspect, the invention provides a treatment method in which glufosfamide, alone or in combination with another anticancer agent is administered in an amount and according to a schedule or administration regimen discovered to be effective for treatment ("therapeutically effective"). Chemotherapy for cancer typically involves multiple "rounds" or "treatment cycles" of administration of one or more drugs, where each cycle comprises administration of the one or more drugs one or more times according to a specified schedule. A cycle is generally (but not necessarily) measured in weeks and can be, for example, 1, 2, 3, 4, 5, 6, 7, or 8 weeks in duration. In some embodiments of the present invention, for example, the treatment cycle is a three-week cycle, a four-week cycle, or a seven-week cycle. In each treatment cycle, a drug is administered at least once and in accordance with a specified schedule,

e.g., daily; once per week; multiple times a week either on consecutive days or non-consecutive days; once every cycle; multiple times every cycle such as for three consecutive days once every three week cycle, and the like. When more than one drug (*e.g.*, two drugs) is administered to a subject, each can be administered according to its own schedule as illustrated above (*e.g.*, weekly; once every three weeks, and the like). Administration of two or more drugs, even those administered with different periodicity, can be coordinated so that both drugs are administered on the same day (at least some of the time) or, alternatively, so the drugs are administered on consecutive days (at least some of the time), for the convenience of the patient and treating physician.

In treatment regimens of the present invention in which glufosfamide and gemcitabine (or another drug) are administered in combination, they can be administered in any order. In certain embodiments, glufosfamide is administered one day before, one day after, or on the same day as the administration of gemcitabine (or another drug). In certain embodiments, gemcitabine (or another drug) is administered on the same day as glufosfamide, and administration of gemcitabine (or another drug) is commenced before, concurrent with, or after administration of glufosfamide. In certain embodiments, gemcitabine (or another drug) is administered (*i.e.* administration will begin) between about 30 minutes to about 4 hours after the administration of glufosfamide. Other administration schedules can be used as generally described herein and more particularly determined by the physician.

Chemotherapeutic drugs are typically administered for from 1 to 8 treatment cycles, or for more cycles (for example, until no further therapeutic benefit is realized from continued treatment). In various embodiments of the present invention, glufosfamide is administered by intravenous infusion to a patient in need of treatment for cancer for 1, 2, 3, 4, 5, 6, 7, 8, or more than 8 treatment cycles, each cycle comprising an infusion of glufosfamide in the range of:

a) about 1.5 to 4.5 to 5.0 to 6.0 to about 8.0 g/m^2 (grams of glufosfamide to surface area of patient) over an infusion period or time of 1, 2, 3, 4, 5, or 6 hours once every three or four weeks; or

b) about 1.5 to 2.0 to about 3.0 g/m^2 over an infusion period of 1, 2, 3, 4, 5, or 6 hours for one to three consecutive days (days 1, 2 and 3) every three or four weeks; or

c) about 0.5 to 1.5 to 1.66 to 2.0 to 2.5 to about 4.0 g/m^2 over an infusion period of 1, 2, 3, 4, 5, or 6 hours once per week.

In accordance with the methods of the invention, and regardless of whether glufosfamide is administered alone or in combination with another anti-cancer agent, glufosfamide is administered at least once per treatment cycle for at least 1, 2, 3, 4, 5, 6, 7, 8, or more than 8 treatment cycles, and in each administration of glufosfamide, glufosfamide is administered to the

cancer patient by intravenous infusion in an amount ranging from 1.5 to about 6.0 g/m^2. In one embodiment, the amount of glufosfamide administered ranges from about 1.5 to about 4.5 g/m^2. In one embodiment, the amount of glufosfamide administered is 4.5 g/m^2. In one embodiment, the amount of glufosfamide administered is 4.5 g/m^2. The glufosfamide infusion can be administered over a period 1, 2, 3, 4, 5, or 6 hours. In one embodiment, the glufosfamide is administered once every three or four weeks (the treatment cycle time) at a dose of 4.5 g/m^2 over an infusion time of 1, 4, or 6 hours.

In one embodiment, glufosfamide is administered so that the glufosfamide concentration in the patient's blood plasma or C_{max} reaches at least 50 micrograms/mL, or at least 100 micrograms/mL, or at least 150 micrograms/mL, or at least 250 micrograms/mL. In one embodiment, these blood plasma levels of glufosfamide are reached by administering glufosfamide in amounts ranging from about 1.5 to 2.5 to about 4.5 g/m^2 over an infusion period of 1 to 4 hours. In one embodiment, glufosfamide is administered so that the glufosfamide AUC is at least 200 micrograms-h/mL, or at least 500 micrograms-h/mL, or at least 1000 micrograms-h/mL. In one embodiment, these blood plasma levels of glufosfamide are reached by administering glufosfamide in amounts ranging from about 1.5 to 2.5 to about 4.5 g/m^2 over an infusion period of 1 to 4 hours.

Glufosfamide is a pro-drug that is converted into the active metabolite isophosphoramide mustard (IPM) *in vivo*, and in one embodiment, glufosfamide or another IPM pro-drug or IPM itself is administered so that the IPM concentration in the patient's blood plasma reaches at least 1 microgram/mL, or at least 3 micrograms/mL, or at least 4 micrograms/mL. In one embodiment, these blood plasma levels of IPM are reached by administering glufosfamide in amounts ranging from about 1.5 to 3.5 to about 4.5 g/m^2, respectively, over an infusion period of 4 hours. In one embodiment, glufosfamide or another IPM pro-drug or IPM itself is administered so that the IPM AUC is at least 10 micrograms-h/mL, or at least 25 micrograms-h/mL, or at least 35 micrograms-h/mL. In one embodiment, these blood plasma levels of IPM are reached by administering glufosfamide in amounts ranging from about 1.5 to 2.5 to about 4.5 g/m^2, respectively, over an infusion period of 1 to 4 hours.

In one embodiment, glufosfamide is administered as described above and gemcitabine is also administered for 1, 2, 3, 4, 5, 6, 7, 8 or more than 8 of the treatment cycles, and each administration of gemcitabine in each treatment cycle comprises an intravenous infusion of gemcitabine in a dose ranging from about 1000 mg/m^2 to about 1500 mg/m^2 to about 2200 mg/m^2 over an infusion time ranging from about 30 to about 150 min.

In one embodiment in which glufosfamide and gemcitabine are co-administered, gemcitabine is administered on weeks 1, 2, 3, 5, 6, and 7 of a 7-week treatment cycle for 1, 2, 3,

4, or more than 4 treatment cycles, and glufosfamide is administered in amounts and frequencies set forth above. In one embodiment, gemcitabine is administered on weeks 1, 2, and 3 of a treatment cycle for 1, 2, 3, 4, 5, 6, or more than 6 treatment cycles, wherein each cycle is a four-week cycle, and glufosfamide is administered in amounts and frequencies set forth above. For example and without limitation, gemcitabine can be administered in accordance with the methods of the invention one day before, one day after, or on the same day as, the administration of glufosfamide. In one embodiment, gemcitabine is administered on the same day as the administration of glufosfamide, about 30 minutes to about 4 hours after the administration of glufosfamide.

In one embodiment in which glufosfamide and gemcitabine are co-administered, gemcitabine is administered so that the gemcitabine concentration in the patient's blood plasma reaches at least 20 micrograms/mL. In one embodiment, this blood plasma level of gemcitabine is reached by administering gemcitabine in an amount of about 1 g/m^2, over an infusion period of about one-half hour. Gemcitabine is a pro-drug that is converted into the active metabolite dFdU *in vivo*, and in one embodiment, gemcitabine is administered so that the dFdU concentration in the patient's blood plasma reaches at least 30 micrograms/mL. In one embodiment, this blood plasma level of dFdU is reached by administering gemcitabine in an amount of about 1 g/m^2 over an infusion period of one-half hour.

As is understood in the art, treatment with cancer therapeutic drugs can be suspended temporarily if toxicity is observed or for the convenience of the patient without departing from the scope of the invention, and then resumed.

In one embodiment, the administration of glufosfamide is initiated after steps have been taken to lower serum glucose levels. This method of the invention is beneficial in that glufosfamide and glucose can compete for the same glucose transporters on the cancer cell, so the lower the concentration of glucose in the blood, the more glufosfamide enters the cancer cell. Blood glucose levels can be lowered by, for example and without limitation, prohibiting the patient from consuming food and glucose containing nutrients for some period of time before the administration of glufosfamide, such as an 8 to 12 to 16 hour period and/or by administering insulin or another agent that lowers blood glucose levels.

In another aspect of the invention, the level of a glucose transporter in the cancer is measured prior to the administration of glufosfamide to determine if the cancer is likely to respond favorably (be treated by) to the administration of glufosfamide. Glucose transporters are believed to transport glufosfamide into the cancer cell, so increased levels of glucose transporters that transport glufosfamide will make a cancer cell more susceptible to glufosfamide therapy.

In this aspect of the invention, a patient or a sample of a patient's tumor is assayed to detect the presence or level of a biological agent that serves as a marker of glucose transport. Methods to determine glucose transporter levels are described in PCT patent publication 2004/081181, incorporated herein by reference. Generally, the higher the level of a glucose transporter, the more likely the cancer will respond favorably to glufosfamide therapy. In one embodiment of the present invention, the level of glucose transporter measured is the level of GLUT7, GLUT8, or GLUT12. Cancers that typically have high levels of these transporters include nasopharyngeal carcinoma, ovarian carcinoma, squamous and adeno lung carcinomas, small cell carcinomas, glioblastoma, chondrosarcoma, esophageal carcinoma, germ cell tumors, and multiple myeloma. Thus, in one embodiment, the present invention provides a method for treating a cancer in a patient, which method first comprises determining that the cancer in the patient expresses a relatively high level of a glucose transporter, including but not limited to GLUT7, GLUT8, or GLUT12. In one embodiment, the level of GLUT1, GLUT2, GLUT3, and/or GLUT4 is determined instead of or in addition to the level of GLUT7, GLUT8, or GLUT12.

A variety of assays can be used to determine the glucose transporter level in a tumor, including assays to measure transporter RNA levels (for example, by quantitative PCR) and antibody assays to measure glucose transporter protein directly. These assays, as well as the PET scan noted below (which also provides a measure of glucose transporter levels), can also be used to monitor the therapeutic effects of glufosfamide administration.

In one embodiment of the invention, a PET scan is used to determine if the cancer to be treated is likely to respond to glufosfamide therapy, alone or in combination with another drug. In this embodiment, PET-scanning is used to determine if the cancer cells take up the PET-scanning agent 2-fluoro-deoxyglucose avidly, and if they do, the physician determines that the cancer is more likely to respond favorably to glufosfamide therapy than cancers that take up the agent less avidly.

Other markers can be used in accordance with the methods of the invention to determine if a patient is likely to respond or is responding favorably to glufosfamide therapy. For example, hypoxia, or low oxygen concentrations, in a tumor typically up-regulates glucose transporter levels. Osteopontin is a serum marker that indicates the presence of hypoxic cells, for example.

As another example, serum carbohydrate antigen 19-9 reportedly can be a useful marker for evaluating the response to gemcitabine therapy in pancreatic cancer (Ziske *et al.*, 2003, *Br. J. Cancer* 89:1413-17). In one embodiment, response to glufosfamide therapy for cancer, wherein glufosfamide is administered as a single agent or in combination with another anticancer agent,

is characterized by measuring the serum carbohydrate antigen 19-9 levels of the subjects during treatment.

As another example, levels of cancer antigen-125 (CA-125) can be used to monitor ovarian cancer. In one embodiment, response to glufosfamide therapy for ovarian cancer, wherein glufosfamide is administered as a single agent or in combination with another anticancer agent, is monitored by measuring the CA-125 level of the subject during treatment, with a reduction in CA-125 indicative of treatment efficacy.

Thus, the present invention provides a variety of methods for the treatment of cancer. In one embodiment, cancer is treated by administering glufosfamide intravenously at a dose of 4.5 g/m^2 over a 1 to 4 hour infusion period once every four weeks, and repeating this administration one or more times, in combination with administering gemcitabine intravenously once a week for 3 weeks out of each 4 week treatment cycle at a dose of 1 g/m^2 over a one-half to one hour infusion period; this embodiment can be used to treat, for example and without limitation, pancreatic cancer, ovarian cancer, and breast cancer. In another embodiment, cancer is treated by administering glufosfamide intravenously at a dose of 4.5 g/m^2 over a 1 to 4 hour infusion period once every three weeks, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, small-cell lung cancer, ovarian cancer, and soft tissue sarcoma. In another embodiment, cancer is treated by administering glufosfamide intravenously at a dose of 1.66 to 2.5 g/m^2 over a 1 to 4 hour infusion period once a week, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, ovarian cancer and soft tissue sarcoma.

In another embodiment, cancer is treated by administering glufosfamide so that the glufosfamide concentration in the patient's blood plasma reaches at least 50 micrograms/mL or 100 micrograms/mL or 150 micrograms/mL, and repeating this administration once every four weeks one or more times, in combination with administering gemcitabine in an amount such that the gemcitabine concentration in the patient's blood plasma reaches at least 20 micrograms/mL, and repeating this administration once a week for 3 weeks out of each 4 week treatment cycle; this embodiment can be used to treat, for example and without limitation, pancreatic cancer, ovarian cancer, and breast cancer. In another embodiment, cancer is treated by administering glufosfamide so that the glufosfamide concentration in the patient's blood plasma reaches at least 50 micrograms/mL or 100 micrograms/mL or 150 micrograms/mL once every three weeks, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, small-cell lung cancer, ovarian cancer, and soft tissue sarcoma. In another embodiment, cancer is treated by administering glufosfamide so that the glufosfamide concentration in the patient's blood plasma reaches at least 150 micrograms/mL or 250

micrograms/mL once a week, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, ovarian cancer and soft tissue sarcoma.

In another embodiment, cancer is treated by administering glufosfamide so that the glufosfamide AUC reaches at least 200 micrograms-h/mL, or at least 500 micrograms-h/mL, or at least 1000 micrograms-h/mL, and repeating this administration once every four weeks one or more times, in combination with administering gemcitabine in an amount such that the gemcitabine concentration in the patient's blood plasma reaches at least 20 micrograms/mL, and repeating this administration once a week for 3 weeks out of the 4 week treatment cycle; this embodiment can be used to treat, for example and without limitation, pancreatic cancer, ovarian cancer, and breast cancer. In another embodiment, cancer is treated by administering glufosfamide so that the glufosfamide AUC reaches at least 200 micrograms-h/mL, or at least 500 micrograms-h/mL, or at least 1000 micrograms-h/mL, once every three weeks, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, small-cell lung cancer, ovarian cancer, and soft tissue sarcoma. In another embodiment, cancer is treated by administering glufosfamide so that the glufosfamide concentration in the patient's blood plasma reaches at least 500 micrograms-h/mL or at least 1000 micrograms-h/mL once a week, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, ovarian cancer and soft tissue sarcoma.

In another embodiment, cancer is treated by administering glufosfamide or another IPM pro-drug or IPM itself so that the IPM concentration in the patient's blood plasma reaches at least 1 microgram/mL, or at least 3 micrograms/mL, or at least 4 micrograms/mL, and repeating this administration once every four weeks one or more times, in combination with administering gemcitabine or another dFdU pro-drug in an amount such that the dFdU concentration in the patient's blood plasma reaches at least 30 micrograms/mL, and repeating this administration once a week for 3 weeks out of each 4 week treatment cycle; this embodiment can be used to treat, for example and without limitation, pancreatic cancer, ovarian cancer, and breast cancer. In another embodiment, cancer is treated by administering glufosfamide or another IPM pro-drug or IPM itself so that the IPM concentration in the patient's blood plasma reaches at least 1 microgram/mL or at least 3 micrograms/mL or at least 4 micrograms/mL once every three weeks, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, small-cell lung cancer, ovarian cancer, and soft tissue sarcoma. In another embodiment, cancer is treated by administering glufosfamide or another IPM pro-drug or IPM itself so that the IPM concentration in the patient's blood plasma reaches at

least 3 to 4 micrograms/mL once a week, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, ovarian cancer and soft tissue sarcoma.

In another embodiment, cancer is treated by administering glufosfamide or another IPM pro-drug or IPM itself so that the IPM AUC reaches at least 10 micrograms-h/mL, or at least 25 micrograms-h/mL, or at least 35 micrograms-h/mL, and repeating this administration once every four weeks one or more times, in combination with administering gemcitabine or another dFdU pro-drug in an amount such that the dFdU concentration in the patient's blood plasma reaches at least 30 micrograms/mL, and repeating this administration once a week for 3 weeks out of the 4 week treatment cycle one or more times; this embodiment can be used to treat, for example and without limitation, pancreatic cancer, ovarian cancer, and breast cancer. In another embodiment, cancer is treated by administering glufosfamide or another IPM pro-drug or IPM itself so that the IPM AUC reaches at least 10 micrograms-h/mL, or at least 25 micrograms-h/mL, or at least 35 micrograms-h/mL, once every three weeks, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, small-cell lung cancer, ovarian cancer, and soft tissue sarcoma. In another embodiment, cancer is treated by administering glufosfamide or another IPM pro-drug so that the IPM AUC in the patient's blood plasma reaches at least 25 micrograms-h/mL or at least 35 micrograms-h/mL once a week, and repeating this administration one or more times; this embodiment can be used to treat, for example and without limitation, ovarian cancer and soft tissue sarcoma.

The present invention having been described in detail in the preceding sections, the following examples are provided to illustrate certain aspects of, but not to limit, the invention.

Example 1

Cell Proliferation Assay

MIA-PaCa-2, AsPC-1, and H766t cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). All cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, sodium pyruvate, nonessential amino acids, L-glutamine, vitamins, and antibiotics. Cells were maintained in a humidified incubator containing 10% CO_2, at 37°C. All chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified. Gemcitabine was purchased from Eli Lilly Co., reconstituted in sterile phosphate-buffered saline (PBS), and stored at room temperature for *in vivo* studies or in aliquots at 20°C for *in vitro* studies. Glufosfamide was prepared as described in U.S. Patent No. 5,622,936 and German Patent No. DE 19534366 and reconstituted in PBS

freshly for each study. The anti-proliferating cell nuclear antigen (PCNA) and anti-CD31 monoclonal antibodies were obtained from Dakocytomation Corporation (Carpinteria, CA). Cells were collected from exponentially growing cultures. Cell numbers were determined by direct counting in a hemocytometer and cell viability by trypan blue exclusion. For cell growth curves, 1 X 10^6 cells were plated in triplicate for each dose level. After 24 hours, the culture medium was removed and replaced with fresh medium mixed with 1 μg/ml of gemcitabine, 10 μg/ml of glufosfamide or both. These concentrations were selected based on preliminary studies and represent approximately 50% inhibitory concentrations. Each day for three days, viable adherent cells were counted directly with a hemocytometer.

Based on preliminary experiments, a concentration of 1 μg/ml of gemcitabine was selected to examine its growth inhibitory effects. At this concentration, gemcitabine inhibited the growth of MiaPaCa-2 and H766t cells but exhibited only moderate effects on the growth of AsPC-1 cells (Figure 1). Glufosfamide at a concentration of 10 μg/ml was less effective than gemcitabine. However, when glufosfamide was added to gemcitabine, additional inhibition of cell growth of MiaPaCa-2 and H766t cells was observed but not the AsPC-1 cells.

Example 2

Determination of DNA Fragmentation by Fluorescence-Activated Cell Sorting (FACS) Analysis

Quantification of DNA fragmentation (apoptosis) was carried out based on the method of Nicoletti *et al.* (1991). 1 x 10^6 cells were incubated with vehicle, gemcitabine (at 1 ug/mL), glufosfamide (at 10 ug/mL), or the combination of both gemcitabine and glufosfamide (at 1 and 10 μg/mL, respectively) for 24 hours. The cells were then collected by gentle trypsinization, washed with PBS, and pelleted by centrifugation. Cells were resuspended in PBS containing 50 μg/ml propidium iodide, 0.1% Triton X-100, and 0.1% sodium citrate. The samples were then were stored at 4°C for 16 hours and vortex-mixed before FACS analysis. The relative percentage of cells in the sub-G1 region was then quantitated and used as an estimate of cells undergoing apoptosis. FACS channels were set based on reference analysis and maintained constant during the experiments.

Because the cytotoxicity assay indicated that glufosfamide and gemcitabine treatment was most effective against the MiaPaCa-2 cells, FACS analysis of DNA fragmentation of MiaPaCa-2 cells was conducted to determine if apoptosis was induced by this combination. Treatment of MiaPaCa-2 cells with 10 μg/mL of glufosfamide or 1 μg/mL of gemcitabine resulted in induction of apoptosis as measured by an increase in apoptosis (sub G1 fraction;

Figure 2) although the effect of gemcitabine was greater. Treatment with both agents resulted in enhanced apoptosis that was greater than additive in nature.

Example 3

Animal Model Testing

Orthotopic tumors were generated using MiaPaCa-2 cells that had been transfected to express red fluorescent protein (RFP) stably as previously described (Katz et al., 2003, *J. Surg. Res.* 113:151-60. Tumor stocks were made by subcutaneously injecting the MiaPaCa-2-RFP cells at a concentration of 5 x 10^6 cells/200 μL into the flanks of nude mice. The tumor tissues were resected aseptically and any grossly necrotic or suspected necrotic or non-RFP tumor tissues were removed. The remaining healthy tumor tissues were subsequently cut into small fragments of a size of approximately 1 mm^3. Recipient mice were anesthetized with isoflurane, and the surgical area was sterilized using iodine and alcohol. An incision approximately 1.5 cm long was made in the left upper abdomen of the nude mouse using a pair of surgical scissors. The pancreas was exposed, and then two pieces of the MiaPaCa-2-RFP tumor fragments were transplanted to the mouse pancreas with 8-0 surgical sutures (nylon) after the capsule of the transplantation site had been stripped. The abdomen was closed with 6-0 surgical sutures (silk). All procedures of the operation were performed with a 7 × magnification microscope (Olympus) under HEPA filtered laminar flow hoods.

During the course of the study, primary tumor size for each animal was followed on a weekly basis. Primary tumor sizes were estimated by measuring the perpendicular minor dimension (W) and major dimension (L) using sliding calipers. Approximate tumor volume was calculated by the formula (W^2x L) x 1/2. At the end of the study, the primary tumor burden was verified and metastasis to major organs evaluated by whole-body imaging as previously described. Briefly, the mice were placed in a fluorescent light box equipped with a fiberoptic light source of 490 nm (Lightools Research, Encinitas, CA) and images obtained with a Leica fluorescence stereo microscope model LZ12 equipped with a mercury lamp and a 50-W power supply. Selective excitation of red fluorescence protein (RFP) was produced through a D425/60 band-pass filter and 470 DCXR dichroic mirror. Emitted fluorescence was collected through a long-pass filter GG475 (Chroma Technology, Brattleboro, VT) on a Hamamatsu C5810 3 chip cooled color charge-coupled device camera (Hamamatsu Photonics, Bridgewater, NJ). Images were processed for contrast and brightness and analyzed with the use of Image Pro Plus 3.1 software (Media Cybernetics, Silver Spring, Maryland). High resolution images of 1024 X 724 pixels were captured directly on an IBM PC or continuously through video output on a high-resolution Sony VCR (model SLV-R1000; Sony, Tokyo).

In preliminary experiments, six mice per group were studied in which glufosfamide was injected, IV, in doses of 3 – 100 mg/kg/day for 14 days. Saline was administered to control mice. Based on the results of this study, doses of 10 and 30 mg/kg/day were selected for a second study. Metastatic frequency of all groups was analyzed with Fisher's exact test. Comparison of animal survival time between each treatment group and untreated control was analyzed using the Log-rank analysis. Comparison of tumor sizes was determined with the Kruskal Wallis test followed by a log rank analysis. All values were considered significant if $P < 0.05$. Figure 3A shows final tumor volumes recorded at necropsy for glufosfamide dosed at 0, 3, 10, 30 and 100 mg/kg, IV, for 14 days, as well as gemcitabine ("Gem") at 300 mg/kg, IP, once a week for three weeks. Treatment was initiated when tumors reached 100 – 150 mm^3. Glufosfamide treatment resulted in dose related reductions in tumor volume; a dose of 100 mg/kg was similar in effect to gemcitabine. In addition, at doses of 30 mg/kg or higher there was a significant increase in the time to 50% survival, an effect not significantly different from that observed with gemcitabine (Figure 3B).

Example 4

Glufosfamide And Gemcitabine Combination Therapy

A combination of glufosfamide and gemcitabine was administered daily for 7 days to female NMRI nu/nu mice that were carrying tumors derived from HS766-T or As-PC-1 human pancreatic cancer cells. Mice were administered doses of vehicle control (0.9% sodium chloride solution, *i.v.*), gemcitabine (*i.p.*), glufosfamide (*i.v.*) or gemcitabine/glufosfamide combinations once daily for 7 consecutive days. A dose of 1.25 mg/kg was used as the standard dose for gemcitabine throughout the study.

In the HS766-T tumor model, administration of 10 mg/kg glufosfamide caused a dramatic delay in the onset of tumor growth compared with the gemcitabine alone or vehicle control groups. Gemcitabine treatment alone had only a marginal inhibitory effect on tumor progression. The combined administration of 10 mg/kg glufosfamide and 1.25 mg/kg gemcitabine resulted in a statistically significant (P=0.009) reduction of the test animals' tumor size at the end of the study period (Day 42) compared with treatment with glufosfamide alone. In the As-PC-1 tumor model, the dose regimens of glufosfamide and gemcitabine, either alone or in combination, had no apparent inhibitory effect on tumor growth. These results demonstrate that the HS766-T tumor model is sensitive to the combined administration of glufosfamide and gemcitabine.

Example 5

Glufosfamide And Gemcitabine Combination Therapy

A combination of glufosfamide and gemcitabine was administered to nude mice that were carrying tumors derived from type MiPaca2 human pancreatic cancer cells. Mice were administered doses of vehicle control, gemcitabine, glufosfamide, or gemcitabine/glufosfamide combinations as tabulated in Table 1 below (10 mice/group). Glufosfamide was administered *i.v.*, daily for 14 days (Groups 2-4 and 7-12). Gemcitabine was administered at 300 mg/kg, *i.p.*, once a week for three weeks (Groups 6 and 10-12). Gemcitabine was administered at 150 mg/kg, *i.p.*, twice a week for three weeks (Groups 5 and 7-9).

Table 1

Group	Glufosfamide (mg/kg)	Gemcitabine (mg/kg)
1*	0	0
2	10	0
3	30	0
4	100	0
5	0	150
6	0	300
7	10	150
8	30	150
9	100	150
10	10	300
11	30	300
12	100	300

* = vehicle.

Groups 3 and 4 exhibited a modest reduction in tumor size. Approximately 50% reduction in tumor size was observed in Group 6. Group 10 was comparable to Group 6. Groups 11 and 12 exhibited a greater reduction in tumor size compared to Group 6 (Group 12 appeared to be toxic). In this experiment, the administration of gemcitabine at 150 mg/kg (Groups 5, 7-9) resulted in rapid deaths of the animals. The combination of 30 mg/kg of glufosfamide and gemcitabine (Group 11) was significantly more effective than gemcitabine alone (Group 6). Figure 4 shows the weekly primary tumor volumes for groups administered 300 mg/kg gemcitabine with or without glufosfamide. The results demonstrate that tumor reduction in animals administered a combination of glufosfamide and gemcitabine was significantly more effective than gemcitabine alone. The same combination of treatments also provided significant improvement in survival.

Example 6

Immunohistochemistry Of Pancreatic Tumors

In a separate experiment, tumors were established in groups of 5 nude mice by intrapancreatic injection of 1 X 10^6 Mia-PaCa-2 cells after a midline incision and laparotomy under isoflurane anesthesia and aseptic conditions. When tumor size was approximately 150 mm^3, treatment was initiated with 30 mg/kg/day of glufosfamide, IV, for 14 days, 300 mg/kg/day of gemcitabine, IP, once a week for 2 weeks (days 1 and 8 of treatment) or both agents. PBS served as the vehicle control. One day after the final glufosfamide dose, tumors were harvested and frozen until analyzed.

Frozen tissue sections were fixed and 5 μm sections imbedded in paraffin were prepared for assay of terminal deoxynucleotidyl transferase (Tdt)-mediated nick end labeling (TUNEL) using a commercial kit (Promega, Madison, WI) according to the manufacturer's instructions. Background reactivity was determined by processing the slides in the absence of Tdt (negative control). Nuclei were stained with propidium iodide for 10 min. Fluorescent bleaching was minimized with an enhancing reagent (Prolong; Molecular Probes, Eugene, OR). Immunofluorescence microscopy was performed with a fluorescent microscope equipped with narrow bandpass excitation filters. Images were captured using a Nikon camera (Photometrics, Tucson, AZ). DNA fragmentation was detected by localized green fluorescence within the nucleus of apoptotic cells. For the quantification of total TUNEL expression, the number of apoptotic events was counted in 10 random 0.159-mm^2 fields at X 100 magnification.

For detection of PCNA and CD31 by immunohistochemistry, paraffin-embedded tissues were mounted on positively charged Superfrost slides (Fisher Scientific, Houston, TX) and dried overnight. Sections were deparaffinized in xylene, followed by treatment with a graded series of alcohol [100%, 95%, and 80% ethanol/double-distilled H_2O (v/v)] and rehydrated in PBS (pH 7.5). To enhance antigen retrieval sections were microwaved for 5 min. For detection of CD31, the paraffin-embedded tissues were treated with pepsin (Biomeda) for 15 min at 37°C and washed with PBS. After exposure to the anti-PCNA or anti-CD31 antibodies followed by washing with PBS, positive reactions were visualized by incubating the slides with stable 3,3-diaminobenzidine for 10–20 min. The sections were rinsed with distilled water, counterstained with Gill's hematoxylin for 30 s, and mounted with Universal Mount (Research Genetics). Control samples exposed to secondary antibody alone showed no specific staining. For the quantification of MVD, 10 random 0.159-mm2 fields at X 100 magnification were captured for each tumor, and microvessels were quantified according to the method described previously (26, 27). For the quantification of PCNA expression, the number of positive cells was quantified in 10 random 0.159-mm2 fields at X 100 magnification.

Figure 5 shows the apoptotic and proliferative indexes derived from TUNEL and PCNA stained sections of tumors from mice treated with saline, glufosfamide (30 mg/kg, IV daily for 2 weeks), gemcitabine (300 mg/kg once a week for 2 weeks), or both agents. Apoptosis occurred at a low level in control mice but was significantly increased by glufosfamide and to a greater degree by gemcitabine. Combination treatment resulted in a level of apoptosis that was significantly greater than that of either agent alone. PCNA staining was significantly greater than control with both gemcitabine and glufosfamide, indicating significant anti-proliferative effects. Combination treatment resulted in significantly greater PCNA staining compared to either treatment alone. CD31 staining failed to reveal any effects of either treatment alone or combination treatment.

Example 7

Combination Therapy of Glufosfamide and Gemcitabine in Patients with Advanced Solid Tumors and Pancreatic Adenocarcinoma

The following example is provided to illustrate treatment of cancer with glufosfamide and gemcitabine combination therapy. A clinical study is conducted to evaluate safety and demonstrate efficacy of glufosfamide in combination with gemcitabine. The pharmacokinetic parameters of glufosfamide (including the active metabolite isophosphoramide mustard, IPM) and gemcitabine (including the active metabolite dFdU) when administered in combination are also evaluated.

A multi-center study is conducted where subjects are divided into two groups: (i) subjects having locally advanced and/or metastatic solid tumors that have been previously treated or for whom there is no effective standard treatment available (Group I); and (ii) subjects having advanced/metastatic pancreatic adenocarcinoma previously untreated with chemotherapy (Group II). The subjects are assigned to cohorts.

The total duration of the study for each subject is up to 27 weeks, including up to 3 weeks prior to dosing (screening period). The treatment cycle is 4 weeks, and glufosfamide is administered on day 1 of every 4-week cycle and gemcitabine is administered on days 1, 8 and 15 of each 4-week cycle. Glufosfamide (1500, 2500, 3500 and 4500 mg/m^2 - one cohort at each dose level) is administered intravenously over 4 hours once every four weeks on day 1 of each cycle. One-quarter of the dose is administered over the first 30 minutes. The remaining three-quarters of the dose are administered over the following three and half hours. Gemcitabine (1000 mg/m^2) is administered weekly intravenously over 30 minutes on days 1, 8 and 15 of every 4-week cycle. On day 1 of each cycle, gemcitabine infusion begins 30 minutes after completion of the glufosfamide infusion. In subjects with stable disease or a complete or partial response after 2

cycles, the treatment is optionally extended for a period of up to 4 additional 4-week cycles or longer. This dosing schedule is shown in Table 2 below:

Table 2

Week (Day)	1 (1)	2 (8)	3 (15)	4 (22)
Glufosfamide	X	-	-	-
Gemcitabine	x	x	X	-

Vital signs, electrocardiograms, clinical laboratory test results, and adverse events are used to assess safety. Tumor assessments, including computed tomography (CT) scans, are performed at baseline and every 8 weeks while subjects are on the study. Pharmacokinetic parameters are determined from plasma concentrations of glufosfamide and gemcitabine obtained at specific time intervals after dosing. Serial blood samples are collected from each subject for determination of plasma concentrations of glufosfamide and its metabolite isophosphoramide (IPM) (0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 4.25, 4.5, 5, 6, 8, 12, 16 and 24 hours after start of glufosfamide infusion on day 1 of cycles 1 and 2) and gemcitabine and its metabolite dFdU (0, 15, 30, 40, 50 minutes and 1, 1.5, 3.5, 7.5, 11.5 and 19.5 hours after start of gemcitabine infusion on days 1 and 8 of cycles 1 and 2). Blood samples are analyzed for glufosfamide/IPM and gemcitabine/dFdU levels. All statistical tests used for the analysis of efficacy and safety data are two-sided and performed at 0.05 level of significance and the 95% confidence interval is computed.

The following pharmacokinetic parameters for glufosfamide/IPM and gemcitabine/dFdU are computed for each subject: time to maximum concentration (T_{max}); maximum peak observed concentration (C_{max}); the magnitude of the slope of the linear regression of the log concentration vs. time profile during the terminal phase (K_{el}); half-life, computed as ln (2)/K_{el} ($T_{½}$); area under the concentration-time curve (AUC_{last}) from Hour 0 through the last quantifiable concentration time (LQCT), where LQCT is the time at which the last sample with a quantifiable concentration was drawn; Area under the concentration-time curve (AUC) from 0 to infinity, computed using the linear trapezoidal rule as $AUC_{last} + C_{LQCT} / K_{el}$; Clearance (Cl) computed as Dose divided by AUC (glufosfamide and gemcitabine only); Apparent steady-state volume of distribution (Vss), computed as the Clearance (Cl) multiplied by $\{[AUMC/AUC^2] - [T_I/2]\}$, where AUMC is the area under the first moment of the plasma concentration time curve (glufosamide and gemcitabine only) and T_I is the duration of infusion; Apparent volume of distribution in the post-distributive phase (V_β) computed as the ratio of Cl to the terminal elimination rate constant, K_{el} (glufosamide and gemcitabine only). Dose-adjusted AUC and C_{max} is calculated for each subject by dividing AUC and C_{max} by dose. Efficacy outcomes are evaluated as determined by response

rate, duration of response, progression-free survival, and overall survival for pancreatic cancer subjects (6- and 12-month survival and change in serum carbohydrate 19-9).

Nineteen patients with pancreatic (8), gall bladder (4), and ovarian (1), breast (1), liver (1), gastric/duodenal (2) and kidney (1) carcinoma and sarcoma (1) have been enrolled. Two DLTs (dose limiting toxicity) have occurred: Grade 3 fatigue at 2500 mg/m^2 and Grade 4 thrombocytopenia at 4500 mg/m^2. Both cohorts were expanded. No DLTs occurred in either the 1500 or 3500 mg/m^2 cohorts. Phase 1 data indicate that glufosfamide in a dose of 4500 mg/m^2 can be safely administered in combination with gemcitabine. Five patients completed all 6 cycles, and one other patient continues on study. Reasons for discontinuation were progressive disease (11), clinical deterioration (1), adverse events (AE) (1), and death (1). Grade 3/4 neutropenia occurred in 7 patients (5 during Cycle 1) and Grade 3/4 thrombocytopenia in 5 patients (2 during Cycle 1). The CrCL fell below 60 mL/min in one patient. One unconfirmed partial response was reported; 9 of 17 (53%) evaluable patients with a response assessment at 8 weeks had stable disease (SD), including one patient with ovarian cancer who completed 8 cycles of therapy; one patient with breast cancer who completed 10 cycles of therapy; and 4 patients with pancreatic cancer who completed at least 7 cycles of therapy.

Pharmacokinetic analyses suggest no interaction between glufosfamide and gemcitabine. As illustrated in Figures 6 and 7, glufosfamide and IPM exhibit linear pharmacokinetics between 1500 - 4500 mg/m^2 with a half-life of approximately 2-3 hours; the pharmacokinetics of glufosfamide and IPM are cycle-independent. Glufosfamide has a large Vd_{ss} (approximately 9-15 L/m^2) and low clearance (80 – 90 mL/min/m^2). Further, as illustrated in Figures 8 and 9, glufosfamide does not affect the pharmacokinetics of gemcitabine and dFdU. Figures 8 and 9 represent the plasma concentration vs. time profile of gemcitabine and dFdU on Days 1 (i.e., in the presence of glufosfamide at all doses) and 8 (in the absence of glufosfamide) of Cycles 1 and 2. The lack of a significant difference in the plasma concentration profiles between Days 1 and 8 is indicative of a lack of effect of glufosfamide (at all the doses since the glufosfamide data from all doses were pooled).

Example 8

Glufosfamide Therapy For Gemcitabine-Refractory Metastatic Pancreatic Adenocarcinoma

The following example is provided to illustrate treatment of gemcitabine-refractory metastatic pancreatic adenocarcinoma with glufosfamide therapy. A multi-center, randomized, open-label study is conducted to evaluate the safety and efficacy of glufosfamide in subjects with gemcitabine-refractory metastatic pancreatic adenocarcinoma as measured by overall survival compared with best supportive care.

Subjects are divided into two groups of 150 subjects per treatment group (i) glufosfamide treatment group (Group I) and (ii) best supportive care (BSC, Group II). Subjects in Group I are administered glufosfamide for a duration of up to 51 weeks. Glufosfamide is administered intravenously over 6 hours once every 3 weeks at 4500 mg/m^2 for up to 17 doses. One-quarter of the dose is infused over 30 minutes and the remainder over the following five and half hours. Subjects may receive palliative radiotherapy but not within ±48 hours of a dose of glufosfamide. To assess the efficacy of glufosfamide treatment, subjects in Group II are not administered any medication that has antitumor effects, e.g., chemotherapy or other systemic cytotoxic/cytostatic agent-mediated therapies. Other appropriate supportive measures and concomitant medications that do not have antitumor effects, such as analgesics, antibiotics, transfusions, hematopoietic colony-stimulating factors (as therapy but not as prophylaxis), erythropoietin, megestrol acetate for appetite stimulation, are administered when appropriate. Subjects in Group I also receive best supportive care. This dosing schedule is shown in Table 3 below.

Table 3

Week	1	2	3
Group I	x	-	-
Group II	-	-	-

Tumor assessment is performed at baseline and every 6 weeks for the first 24 weeks and then every 9 weeks until disease progression are documented. Pharmacokinetic samples are collected from subjects in Group I during cycles 1 and 2. Blood samples for plasma concentrations of glufosfamide/IPM are collected at the following times on day 1 of cycles 1 and 2 from the subjects in Group I at the following time points: pre-dose and immediately before completion of glufosfamide infusion. Additional pharmacokinetic parameters are measured for a subset of 24 subjects in Group I (AUC, C_{max}, and $T_{½}$ for glufosfamide/IPM). Blood samples are collected from this subset at the following times on day 1 of cycles 1 and 2: pre-dose, 0.5 (immediately before changing the infusion rate), 1, 3, 6 (immediately before completion of glufosfamide infusion), 6.25, 6.5, 7, 8, 10, 16, 24 hours after the start of glufosfamide infusion.

The pharmacokinetic parameters (as described in Example 7 above) for glufosfamide/IPM (day 1 of cycles 1 and 2) are computed for each subject in the 24-subject subset. Efficacy outcomes are evaluated based on the response rate (complete response and partial response), duration of response, progression-free survival, 6- and 12-month survival, changes in VAS pain score and serum carbohydrate 19-9 response compared with best supportive care. The primary efficacy hypothesis tested by the study is that glufosfamide treated subjects with gemcitabine-refractory metastatic pancreatic adenocarcinoma have improved overall survival compared with best supportive care.

Example 9

Glufosfamide Therapy For Small-Cell Lung Cancer (SCLC); Extensive Recurrent Sensitive SCLC; Ovarian Cancer; Soft Tissue Sarcoma and Advanced Soft Tissue Sarcoma

Ovarian cancer, small-cell lung cancer, extensive recurrent sensitive SCLC, soft tissue sarcoma and advanced soft tissue sarcoma cancers are treated in accordance with the methods of the invention by intravenous administration of glufosfamide over an infusion time of one hour at a dose of 4500 mg/m^2 or a dose of 5000 mg/m^2 administered once every 3 weeks.

In one embodiment the patient has small-cell lung cancer and has previously received ICE (ifosfamide/carboplatin/etoposide) or ICE-V (ifosfamide/carboplatin/etoposide/vincristine) therapy. In one embodiment the patient has small-cell lung cancer and is treated with glufosfamide concurrently with (i.e., in combination with) ICE or ICE-V therapy.

Optionally, ovarian cancer patients are treated by intravenous administration of glufosfamide over an infusion time of one hour at a dose of 1600 mg/m^2, 2500 mg/m^2 or 5000 mg/m^2 administered once a week.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes can be made and equivalents can be substituted without departing from the scope of the invention. In addition, many modifications can be made to adapt a particular situation, material, composition of matter, process, process step or steps, to achieve the benefits provided by the present invention without departing from the scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an indication that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same.

CLAIMS

1. A method of treating cancer, which method comprises administering glufosfamide to a cancer patient by intravenous infusion in an amount ranging from 1.5 to about 6.0 g/m^2 for an infusion time ranging from one to six hours, wherein said administering step is conducted at least once every four weeks and is repeated at least once.

2. The method of Claim 1, wherein said amount of glufosfamide administered in said administering step is 4.5 g/m^2 and said administering step is conducted at least once every three weeks.

3. The method of Claim 1, wherein said amount of glufosfamide administered in said administering step is from 1.5 to 3.0 g/m^2 and said administering step is conducted at least once a week.

4. A method of treating cancer, which method comprises administering glufosfamide to a cancer patient in an amount sufficient to produce a glufosfamide concentration in said patient's blood plasma of at least 50 micrograms/mL.

5. The method of Claim 4, wherein said glufosfamide concentration is at least 100 micrograms/mL.

6. The method of Claim 4, wherein said glufosfamide concentration is at least 150 micrograms/mL.

7. A method of treating cancer, which method comprises administering glufosfamide to a cancer patient in an amount sufficient to produce a glufosfamide AUC of at least 200 micrograms-h/mL.

8. The method of Claim 7, wherein said glufosfamide AUC is at least 500 micrograms-h/mL.

9. The method of Claim 7, wherein said glufosfamide AUC is at least 1000 micrograms-h/mL.

10. A method of treating cancer, which method comprises administering glufosfamide or another pro-drug of isophosphoramide mustard (IPM) or IPM itself to a cancer patient in an amount sufficient to produce an IPM concentration in said patient's blood plasma of at least 1 microgram/mL.

11. The method of Claim 7, wherein said IPM concentration is at least 3 micrograms/mL.

12. The method of Claim 7, wherein said IPM concentration is at least 4 micrograms/mL.

13. A method of treating cancer, which method comprises administering glufosfamide or another pro-drug of isophosphoramide mustard (IPM) or IPM itself to a cancer patient in an amount sufficient to produce an IPM AUC in said patient's blood plasma of at least 10 micrograms-h/mL.

14. The method of Claim 13, wherein said IPM AUC is at least 25 micrograms-h/mL.

15. The method of Claim 13, wherein said IPM AUC is at least 35 micrograms-h/mL.

16. The method of any of Claims 1 – 15, wherein gemcitabine is also administered by an intravenous infusion for an infusion time ranging from about 30 to about 150 min., and each administration of gemcitabine is in an amount ranging from about 1000 mg/m^2 to 2200 mg/m^2.

17. The method of any of Claims 1 – 15, wherein gemcitabine is also administered by an intravenous infusion for an infusion time ranging from about 30 to about 150 min., and each administration of gemcitabine is in an amount sufficient to produce a gemcitabine concentration in said patient's blood plasma of at least 20 micrograms/mL.

18. The method of any of Claims 1 – 15, wherein gemcitabine is also administered by an intravenous infusion for an infusion time ranging from about 30 to about 150 min., and each administration of gemcitabine is in an amount sufficient to produce a dFdU concentration in said patient's blood plasma of at least 30 micrograms/mL.

19. The method of any of Claims 1 – 18, in which said cancer is selected from the group of cancers consisting of an advanced and/or metastatic malignancy that has not been previously treated or has been previously treated with surgery, radiation, or chemotherapy; breast cancer; colorectal cancer; metastatic colorectal cancer; non-Hodgkins lymphoma; ovarian cancer; pancreatic cancer; chemotherapy-refractory pancreatic cancer; gemcitabine-refractory pancreatic cancer; sarcoma; PET-positive sarcoma; and small-cell lung cancer (SCLC).

20. The method of any of Claims 1 – 19 in which an agent with antitumor activity selected from the group consisting of bevacizumab (Avastin®), carboplatin, cetuximab (Erbitux®), cisplatin, dacarbazine (DTIC), 2-deoxyglucose, doxorubicin (*e.g.*, Doxil® and Caelyx®), EGFR inhibitors (e.g., Iressa), erlotinib (Tarceva®), etoposide, exatecan, imatinib mesylate (Gleevec®), irinotecan, methotrexate, Panvac-VF®, pemetrexed (Alimta®), rituximab, rubitecan (Orathecin®), taxanes (*e.g.*, docetaxel and paclitaxel), topotecan, vincristine, and trastumab (Herceptin®), is also administered to said patient.

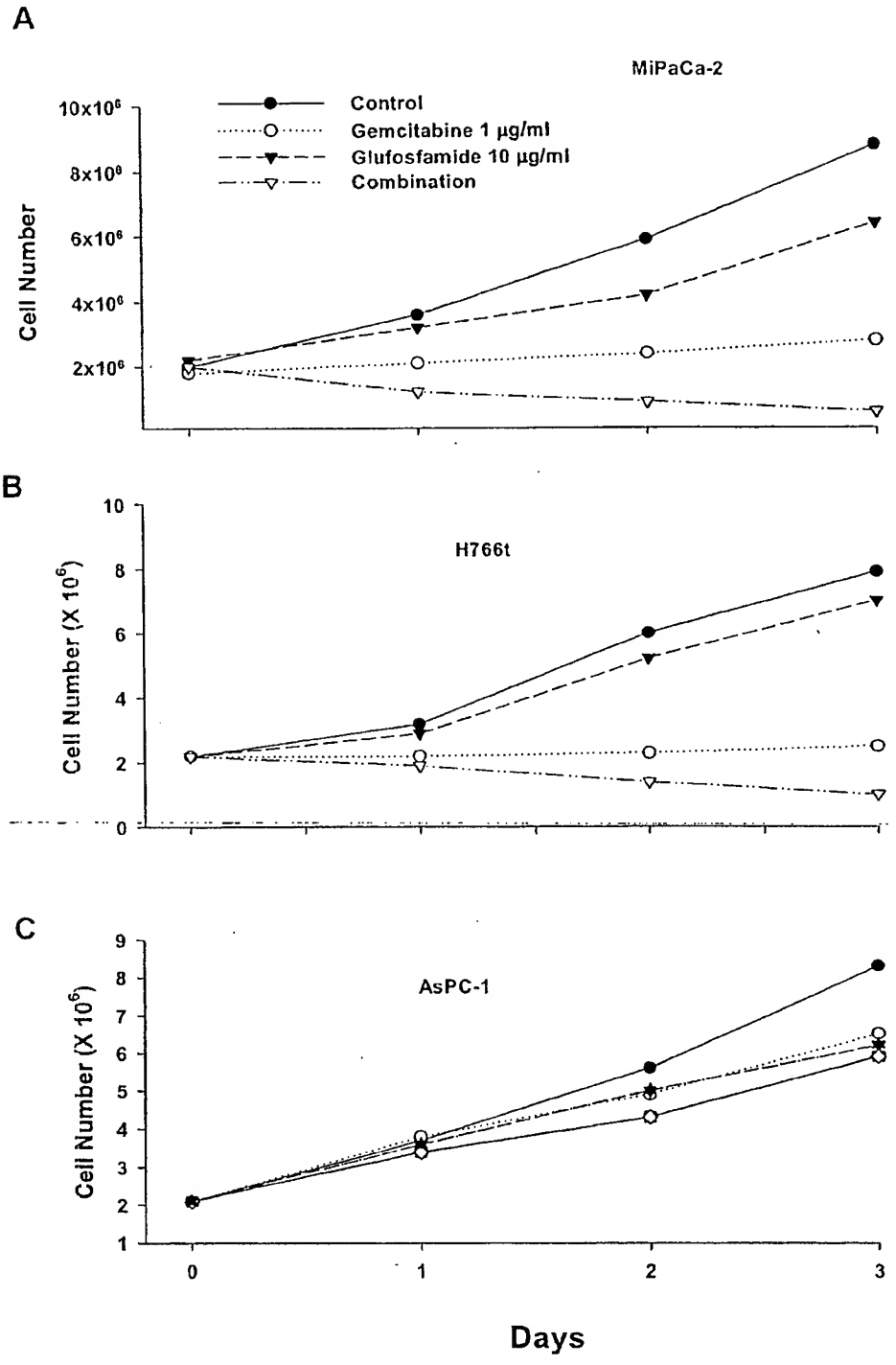
21. The method of any of Claims 1 – 20, wherein said patient is selected for initial or continuing treatment with glufosfamide based on a test conducted to determine or infer a level of a glucose transporter in a cancer cell or tissue of said patient.

22. The method of Claim 21, wherein said test measures a level of a glucose transporter selected from the group consisting of GLUT1, GLUT2, GLUT2, GLUT4, GLUT7, GLUT8, or GLUT12.

23. The method of Claim 21, wherein said test is a PET scan.

24. The method of Claim 21, wherein said test determines or infers a level of hypoxia in a cancer tissue of said patient.

Figure 1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MiPaCa-2
Control
Gemcitabine 1 µg/ml
Glufosfamide 10 µg/ml
Combination
Cell Number
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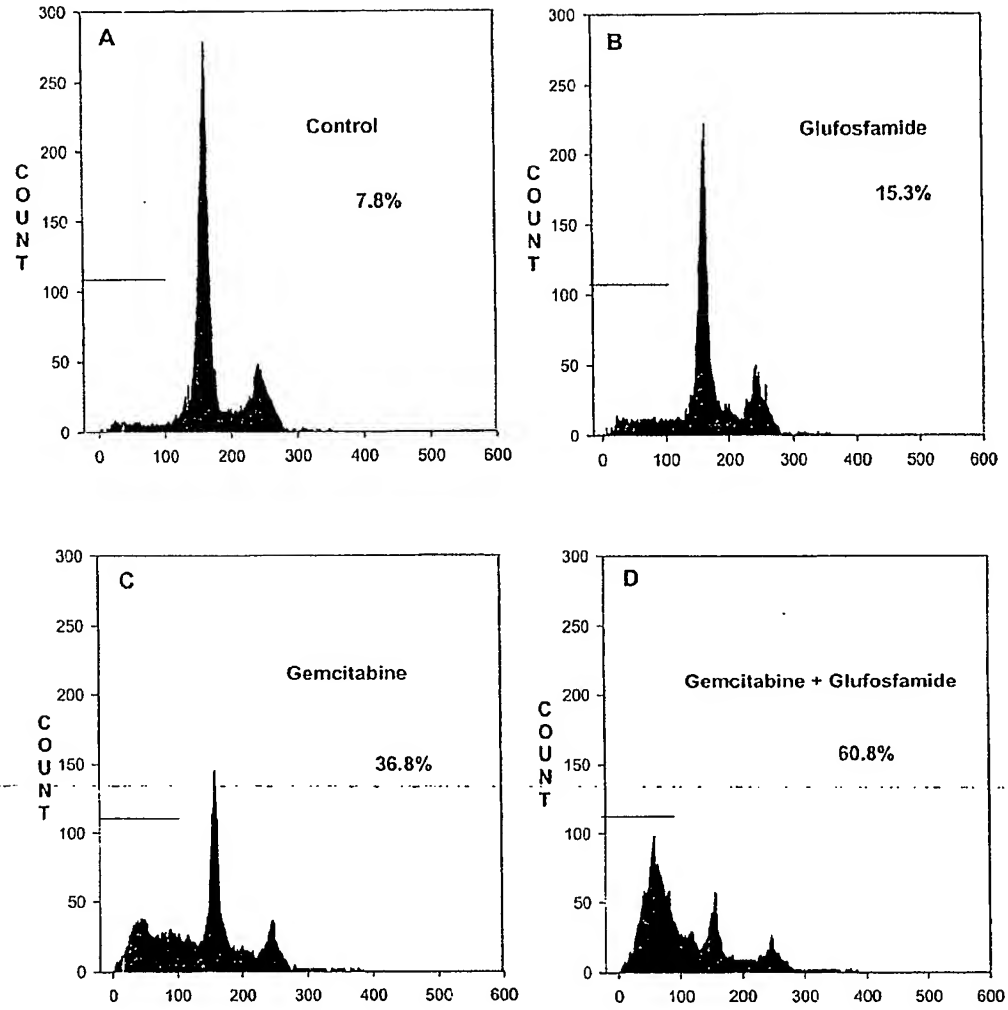
8x10⁶
6x10⁶
4x10⁶
2x10⁶
B
H766t
Cell Number (X 10⁶)
10
8
6
4
2
0
C
AsPC-1
Cell Number (X 10⁶)
9
8
7
6
5
4
3
2
1
0
1
2
3
Days

Figure 2:


A
Control
7.8%
B
Glufosfamide
15.3%
C
Gemcitabine
36.8%
D
Gemcitabine + Glufosfamide
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COUNT
0
50
100
150
200
250
300
0
100
200
300
400
500
600

Figure 3

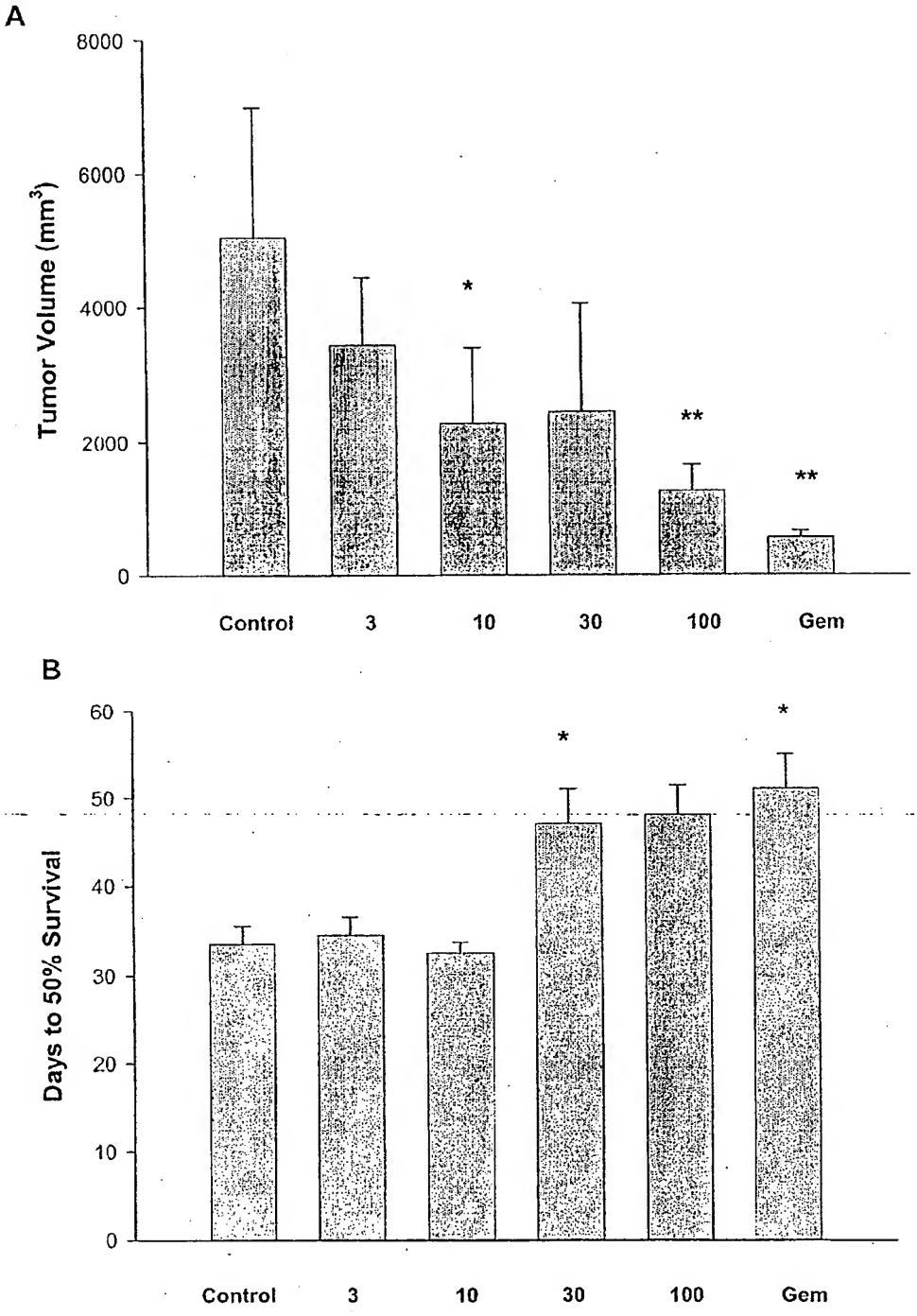
A
8000
6000
4000
2000
0
Tumor Volume (mm3)
*
**
**
Control
3
10
30
100
Gem
B
60
50
40
30
20
10
0
Days to 50% Survival
*
*
Control
3
10
30
100
Gem

Figure 4:

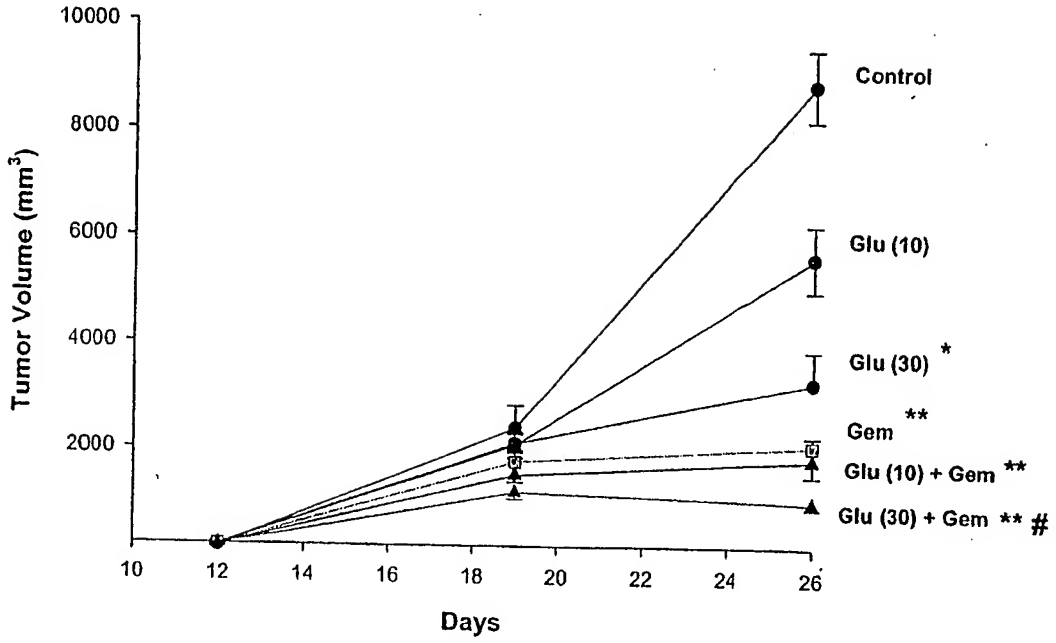
10000
8000
6000
4000
2000
Tumor Volume (mm3)
10
12
14
16
18
20
22
24
26
Days
Control
Glu (10)
Glu (30) *
Gem **
Glu (10) + Gem **
Glu (30) + Gem ** #

Figure 5

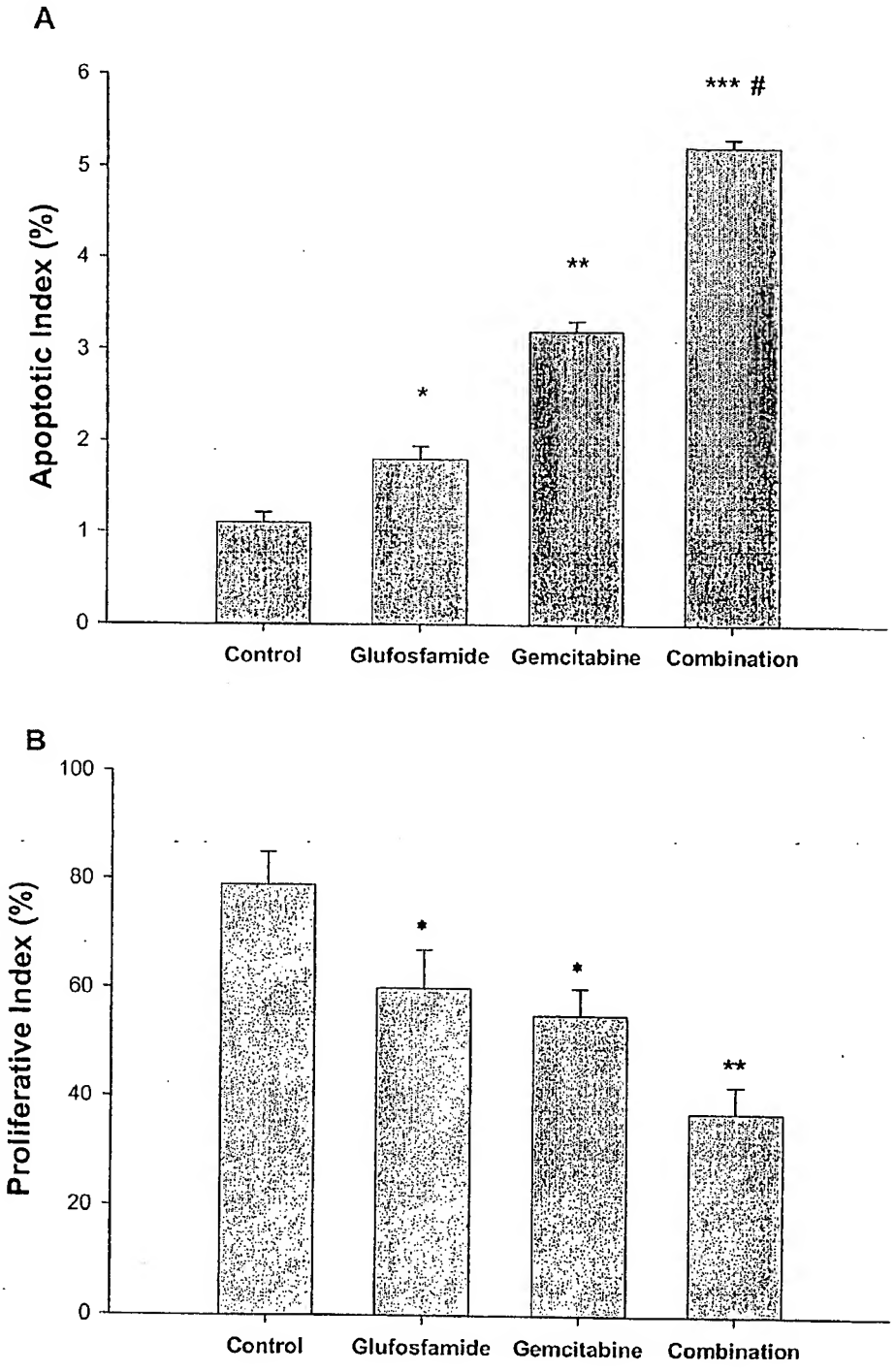
A
Apoptotic Index (%)
0
1
2
3
4
5
6
*
**
*** #
Control
Glufosfamide
Gemcitabine
Combination
B
Proliferative Index (%)
0
20
40
60
80
100
*
*
**
Control
Glufosfamide
Gemcitabine
Combination

Figure 6: Glufosfamide Pharmacokinetics

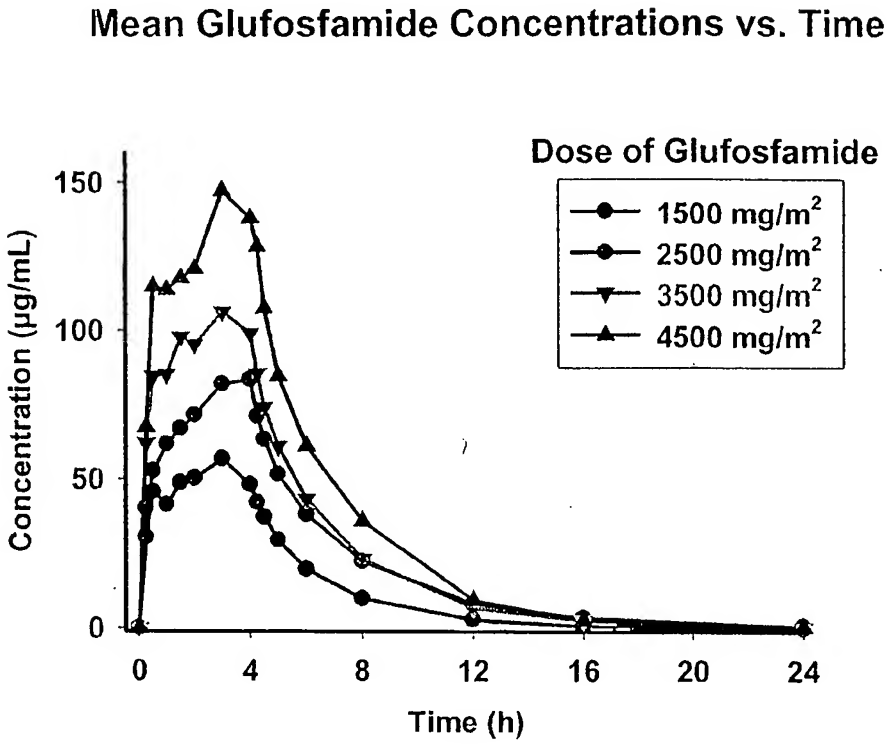

Figure 7: Isophosphoramide Mustard (IPM) Pharmacokinetics

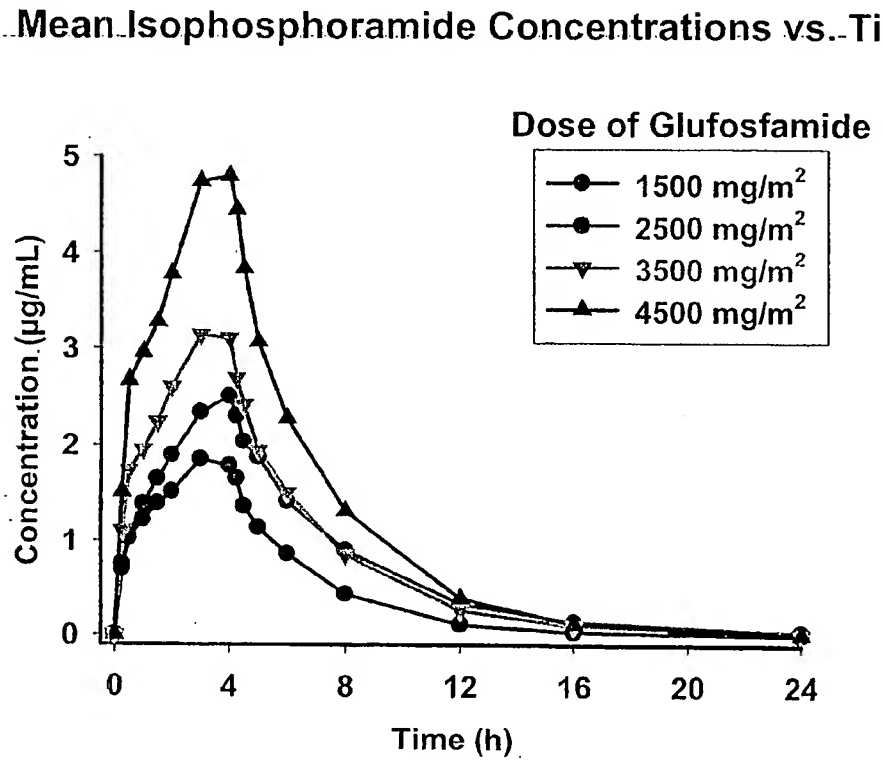

Figure 8: Gemcitabine Pharmacokinetics

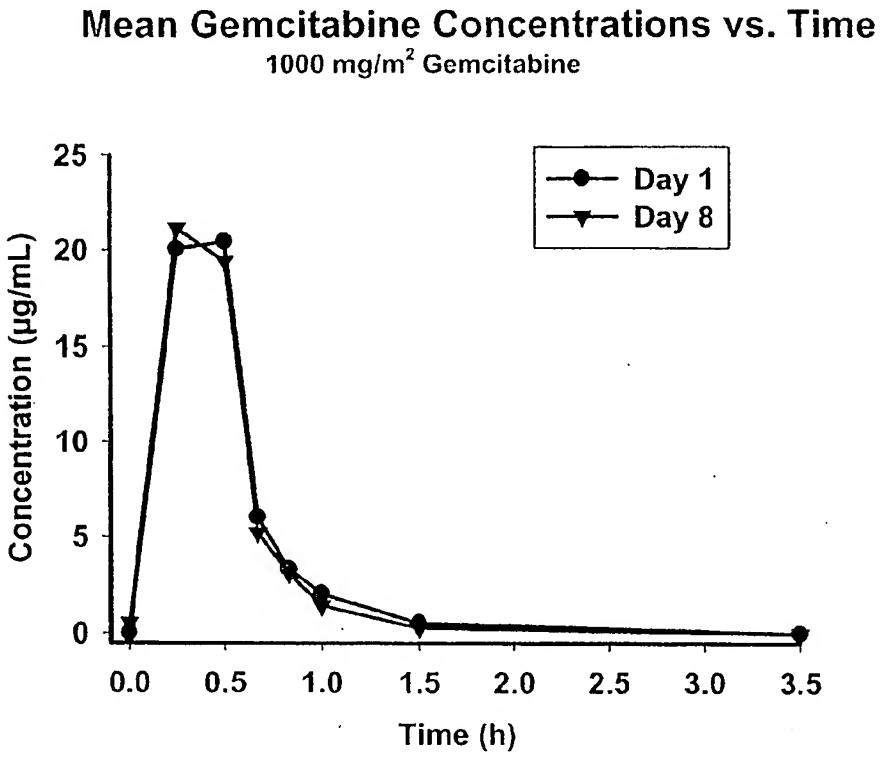

Figure 9: dFdU Pharmacokinetics